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ORIGINAL ARTICLE

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The Effects of Type 1 Diabetes and Diabetic Peripheral Neuropathy on the Musculoskeletal System: A Case–Control Study

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ABSTRACT

Fracture risk is increased in type 1 diabetes (T1D). Diabetic neuropathy might contribute to this increased risk directly through effects on bone turnover and indirectly through effects on balance, muscle strength, and gait. We compared patients with T1D with (T1DN+, $n = 20$) and without (T1DN−, $n = 20$) distal symmetric sensorimotor polyneuropathy and controls ($n = 20$). We assessed areal bone mineral density (aBMD) and appendicular muscle mass by dual-energy X-ray absorptiometry, microarchitecture by high-resolution peripheral quantitative tomography at the standard ultra-distal site and at an exploratory 14% bone length site at the tibia and radius, bone turnover markers, and muscle strength, gait, and balance by Short Physical Performance Battery (SPPB). At the standard ultra-distal site, tibial cortical porosity was 56% higher in T1DN+ compared with T1DN− ($p = .009$) and correlated positively with the severity of neuropathy (Toronto Clinical Neuropathy Score; $r = 0.347$, $p = .028$) and negatively with nerve conduction amplitude and velocity ($r = −0.386$, $p = .015$ and $r = −0.358$, $p = .025$, respectively). Similar negative correlations were also observed at the radius ($r = −0.484$, $p = .006$ and $r = −0.446$, $p = .012$, respectively). At the exploratory 14% offset site (less distal), we found higher trabecular volumetric BMD (tibia 25%, $p = .024$; radius 46%, $p = .017$), trabecular bone volume (tibia 25%, $p = .023$; radius 46%, $p = .017$), and trabecular number (tibia 22%, $p = .014$; radius 30%, $p = .010$) in T1DN− compared with controls. Both CTX and PINP were lower in participants with TD1 compared with controls. No difference was found in aBMD and appendicular muscle mass. T1DN+ had worse performance in the SPPB compared with T1DN− and control. In summary, neuropathy was associated with cortical porosity and worse performance in physical tests. Our findings suggest that bone structure does not fully explain the rate of fractures in T1D. We conclude that the increase in the risk of fractures in T1D is multifactorial with both skeletal and non-skeletal contributions. © 2021 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: TYPE 1 DIABETES MELLITUS; BONE MICROARCHITECTURE; DIABETIC NEUROPATHY; BONE TURNOVER MARKERS; SHORT PHYSICAL PERFORMANCE BATTERY

Introduction

Diabetes is a chronic disease characterized by hyperglycemia. In type 1 diabetes (T1D), the hyperglycemia is caused by β-cells autoimmune destruction, leading to insulin deficiency.[1] Juvenile onset is considered typical of T1D, but people of any age can be affected and up to 50% of cases start in adulthood.[1] Most people living with T1D are adults.[1] Diabetic neuropathy is a result of nerve damage and leads to sensory abnormalities.[2] Diabetic neuropathy can cause negative symptoms such as impaired touch, vibration, pinprick, hot and cold sensation, or positive symptoms such as paradoxical pain and hypersensitivity.[2] Distal symmetrical sensorimotor polyneuropathy (DSPN) is the most common form of diabetic neuropathy and has a stocking–glove distribution.[2]

Diabetes is a recognized risk factor for fractures.[3] A number of meta-analyses have reported an increased risk of fractures in people with diabetes.[4–10] The risk varies according to the type of the disease and the skeletal site and is higher in people with T1D. The most recent meta-analysis reported an almost fivefold increase in the risk of hip fractures in T1D and a 33% increase in type 2 diabetes (T2D).[10] Previous analysis found a 15% decrease in the risk of wrist fractures and a 30% increase in the risk of ankle fractures, but data came mainly from T2D patients.[9] Data specifically on T1D reported a non-significant increase in wrist fractures risk (hazard ratio [HR] = 1.78; 95% confidence

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interval [CI] 0.91–3.47) and an increase in ankle fractures (HR = 2.56; 95% CI 1.41–4.61).[11]

The mechanisms for increased fracture risk are not fully established, but it is likely to be multifactorial. Dual-energy X-ray absorptiometry (DXA) can predict fractures in diabetes.[12] Overall, bone mineral density (BMD) is decreased in people with T1D,[5] but the small decrease in BMD does not explain the magnitude of the increase in the risk of fractures.[5] Several studies have investigated bone microarchitecture in diabetes using high-resolution peripheral quantitative computed tomography (HR-pQCT).[13–19] Most of the studies have addressed T2D[14–20] and data on T1D are scarce.[13] Results are conflicting, and the most common finding was an increase in the cortical porosity reported in T2D[14–18,20] but not in T1D.[13]

Some studies have reported differences in microarchitecture associated with diabetic microvascular disease (MVD).[13,14] Although neuronal regulation of bone metabolism has been described,[21,22] no study has investigated the effect of diabetic neuropathy on the skeleton. DSPN is a distal symmetrical predominantly sensory neuropathy that affects up to 50% of patients with diabetes.[23] The resulting sensory ataxia might also affect balance and increase the risk of falling.[24–26] We investigated the effect of T1D and diabetic neuropathy on the skeleton. We hypothesized that neuropathy would be associated with cortical porosity and poor physical performance in T1D. The aim of this study is to compare bone structural and biochemical analysis, appendicular muscle mass, and physical function test between adults with T1D diabetes with and without DSPN and controls.

Materials and Methods

Participants and methods

This was a single-center, observational, cross-sectional, case-controlled study to evaluate the effects T1D and diabetic neuropathy on the skeleton in patients with T1D. White participants with T1D were recruited from diabetes clinics and from research participant lists in Sheffield (UK) between October 2017 and October 2018. They were older than 18 years, had T1D for more than 5 years, and estimated glomerular filtration rate (eGFR) >60 mL/min/1.73m^2. Healthy volunteers were recruited from research participant lists or through emails sent to hospital staff. Exclusion criteria were conditions that preclude analysis or interpretation of scans; history of or current conditions known to affect musculoskeletal health, diabetes, and/or neuropathy assessment or bone metabolism; the use of medications or treatment known to affect musculoskeletal health, diabetes, and neuropathy assessment or bone metabolism, including depot medroxyprogesterone or the combined oral contraceptive pill; alcohol intake greater than 21 units per week; high- or low-trauma fracture less than 1 year before recruitment; and women currently pregnant, trying to conceive, having delivered her last child less than 1 year before recruitment, or in the perimenopausal period including 5 years after menopause.

Neuropathy assessment

All subjects underwent detailed clinical and neurophysiological assessments. The presence of DSPN was defined per Toronto Diabetic Neuropathy Expert Group.[2] We used the Toronto Clinical Neuropathy Score (TCNS) and nerve conduction assessment by DPNCheck (Neurometrix, Waltham, MA, USA) to assess DSPN. The TCNS assesses symptoms, reflexes, and sensory test at the limbs.[2] Abnormalities are graded by scores to a maximum of 19. One examiner (TV) assessed symptoms (foot pain, numbness, tingling, weakness, ataxia, and upper limb symptoms; present 1, absent 0), sensory tests (pinprick, temperature, light touch, vibration, and position; abnormal 1, normal 0), and reflexes (knee and ankle; absent 2, reduced 1, or normal 0). Patients were categorized according to the scoring as no neuropathy (0–5), mild neuropathy (6–8), moderate neuropathy (9–12), and severe neuropathy (>12). The TCNS has been validated against nerve conduction velocities and amplitudes[27] and morphological criteria of sural nerve fiber density.[28]

Sural nerve conduction assessment was performed in both feet using the validated DPNCheck device to confirm the presence of DPN.[29] This is a point-of-care device that assesses nerve amplitude potential (sural nerve action potential [μV]) and conduction velocity (m/s) using principles similar to the standard nerve conduction studies.[30] DPNCheck has been validated and demonstrated excellent reliability and acceptable accuracy in DSPN.

Participants with TCNS score ≤5 and normal nerve conduction assessment were considered without neuropathy (T1DN$^-$; n = 20), while participants with neuropathy (T1DN$^+$; n = 20) were defined by a combination of TCNS score >6 and abnormal nerve conduction.[2] Individuals with HbA1c levels less than 5.7% (39 mmol/mol)[31] were recruited as controls (n = 20). Groups were matched by age, sex, height, and body mass index (BMI).

This study was approved by Liverpool Research Ethics Committee (IRAS 222726, 17/NW/0291). All participants provided written informed consent, in accordance with Good Clinical Practice guidelines.

Areal BMD

DXA was used to measure whole body (coefficient of variation [CV] = 1.3%), lumbar spine (CV = 1.6%), femoral neck (CV = 2.9%), and total hip (CV = 1.5%) areal BMD (aBMD; Discovery A, Hologic Inc., Bedford, MA, USA). Appendicular skeletal muscle mass was determined by DXA and divided by squared height to calculate the appendicular skeletal muscle mass index (ASMI).

HR-pQCT

We used HR-pQCT by XtremeCT I (Scanco Medical AG, Brüttisellen, Switzerland) to assess bone geometry, volumetric BMD (vBMD), microarchitecture, and estimated bone strength at the nondominant radius and tibia in all participants.

Standard ultra-distal site

Image acquisition, analysis, and validation of the method for the standard ultra-distal site was performed as previously described.[32] In summary, the first image was obtained at 9.5 and 22.5 mm from a line placed at the inflection point on the endplate of the distal radius or tibial plafond, respectively[33] (Figs. 1*A* and 2*A*).

Exploratory 14% offset site

An additional exploratory site, located at 14% of the bone length (exploratory 14% offset site) was also scanned (Figs. 1*A* and 2*A*) to further investigate the cortical compartment since previous literature reported findings in cortical porosity in diabetes. One examiner (MP) measured the limb length; for the radius length, with the forearm flexed to 90°, we measured from the tip of the olecranon to the radial styloid process, and for the tibia, with the knee at 90°, we measured from the medial condyle to the

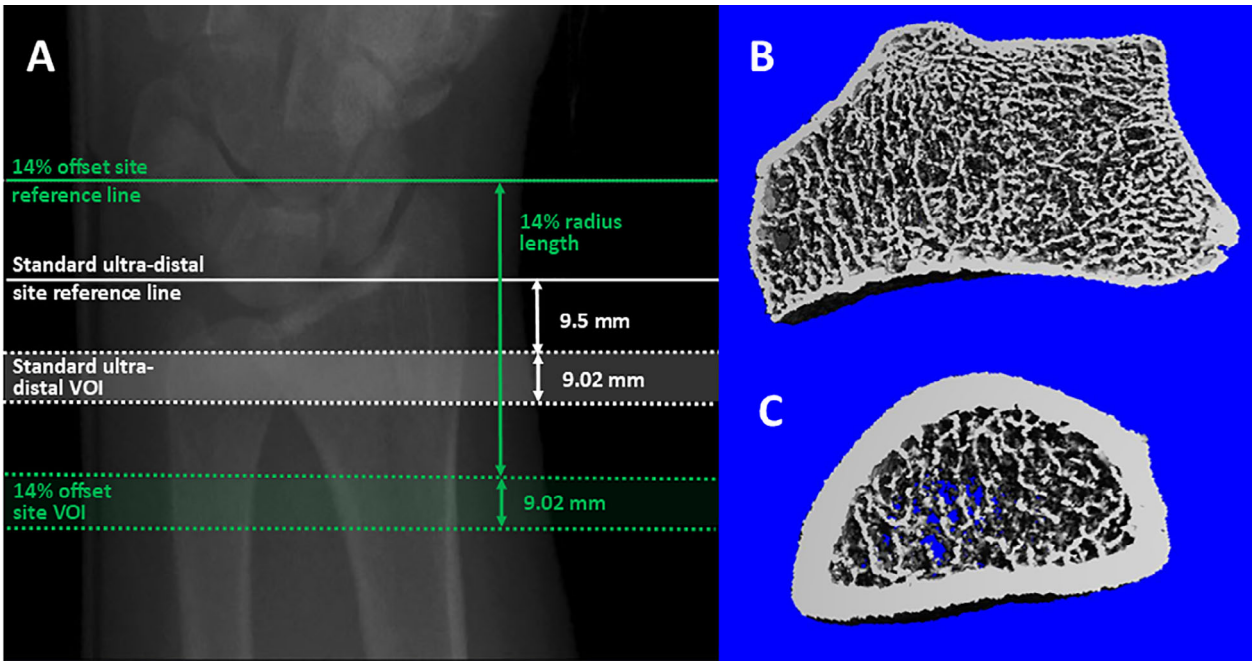

Fig 1. (*A*) Radius scout view scan with reference lines (solid) and the volume of interest (VOI; between two broken lines). In white is the standard ultra-distal site with reference line placed at the inflection point on the endplate of the distal radius plafond. In green, the reference line is placed at the distal end of the radius and the VOI starting at the 14% radius length previously calculated and manually inserted. (*B*) 3D image of the standard ultra-distal radius. (*C*) 3D image of the exploratory14% offset site showing a thick cortex and abundant trabecular bone.

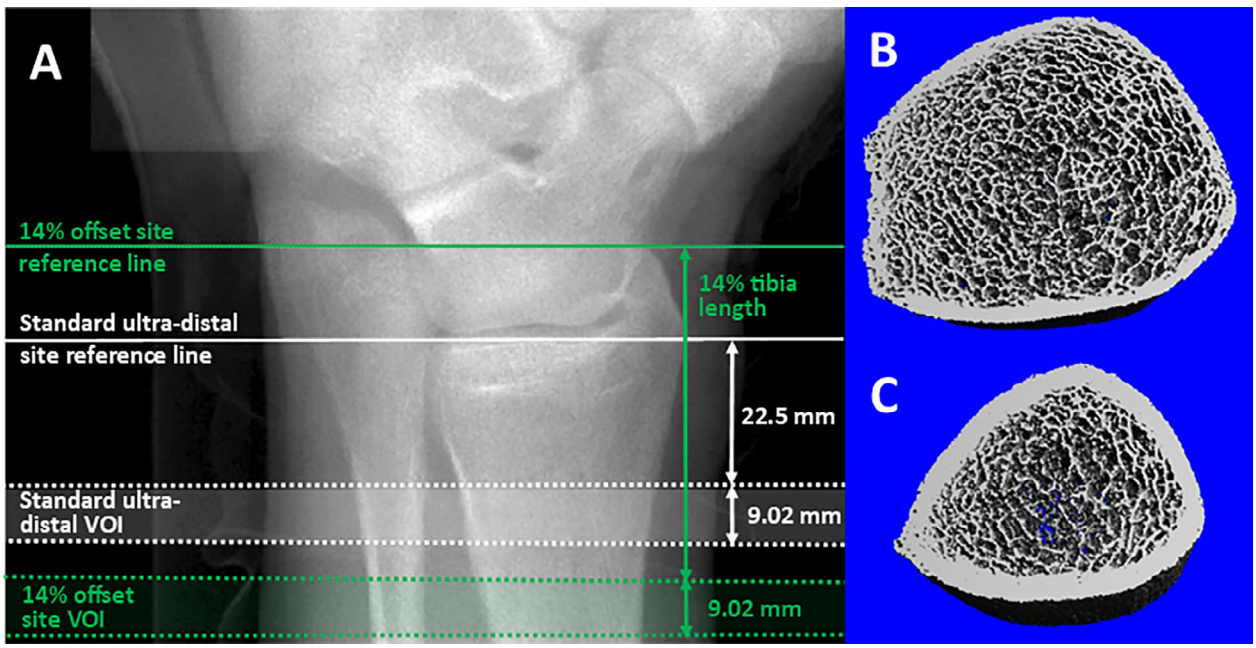

Fig 2. (*A*) Tibia scout view scan with reference lines (solid) and the volume of interest (VOI; between two broken lines). In white is the standard ultra-distal site with reference line placed at the inflection point on the endplate of the distal tibia plafond. In green, the reference line is placed at the distal end of the tibia and the VOI starting at the 14% tibia length previously calculated and manually inserted. (*B*) 3D image of the standard ultra-distal tibia. (*C*) 3D image of the exploratory 14% offset site showing a thick cortex and abundant trabecular bone.

medial malleolus to the nearest 0.1 cm using a tape measure. Each participant's radius and tibia 14% length were calculated and inserted in the relative position to scout view reference line, ensuring that the first slice of the measurement was acquired 1 mm proximal to the 14% site. The scan was then precalibrated before the participant's limb was positioned within the scanner. At all sites, a total of 110 cross-sectional images were obtained, corresponding to 3D representation of 9.02-mm-thick cross sections with an isotropic image voxel size of 82 μm. To assess the quality of the images, we used the visual grading system reported by Engelke and colleagues.[34] In brief, the quality of each scan image was categorized as either perfect (G1) or showing a slight (G2), moderate (G3), or unacceptable (G4) degree of movement artifact. Images G4 were excluded from the analysis.

HR-pQCT image segmentation and analysis were performed using the standard built-in software (version 6.0, Scanco Medical). We analyzed trabecular variables (trabecular bone volume fraction [BV/TV], number [Tb.N], thickness [Tb.Th], and separation [Tb.Sp]) as described.[35] For the standard ultra-distal site, precision errors were calculated in accordance with the International Society for Clinical Densitometry recommendations.[36] In our center, smaller precision errors were observed for densitometric (CV = 0.2–5.5%) than for microstructural (CV = 1.2–7.0%), extended cortical bone (CV = 3.4–20.3%), and biomechanical (CV = 0.3–9.9%) measures at both the radius and tibia.[32]

We used Image Processing Language (IPL v5.08b) provided by the manufacturer (Scanco Medical AG) for cortical variables analysis. Contours that delineated bone from soft tissue and trabecular from cortical bone were automatically placed and manually corrected if needed to define cortical compartment. Cortical bone volume, cortical vBMD, and cortical area were measured, and cortical porosity was calculated.[37,38] We analyzed trabecular and cortical variables at both sites. Micro–finite element analysis was used to estimate biomechanical parameters at standard ultra-distal and exploratory 14% offset sites at the radius and tibia (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland).

We applied micro–finite element analysis (version 1.13; Scanco Medical AG) to the HR-pQCT images to assess bone biomechanical properties. We obtained measures of stiffness and ultimate failure load. The model parameters were set as: material properties isotropic and elastic, cortical bone Young's modulus 20 GPa, trabecular bone Young's modulus 17 GPa, Poisson's ratio 0.3. The proximal end of the section was fixed and a compression strain of 1% was applied to the distal surface of the section.[39]

BTM

Fasting blood samples were collected between 8:00 and 10:00 a.m., processed, and the serum was stored at −80° until analysis. Carboxy-terminal cross-linking telopeptides of type I collagen (CTX) and N-terminal propeptide of type I collagen (PINP) were measured in serum in a single batch using the IDS-iSYS multidisciplined automated chemiluminescence immunoassay (Immunodiagnostic Systems, Boldon, UK). The interassay CVs were 6.5% for CTX and 7.2% for intact PINP, as previously published from our center.[40]

Physical performance test

The Short Physical Performance Battery (SPPB) assesses balance, lower extremity strength, and gait.[41] Participants were asked to stand with their feet side-by-side, in semi-tandem and tandem, to evaluate standing balance, to stand up and sit down five times as quickly as possible, and to "walk at their usual speed" on an 8-foot walking course. Each of these three objective measures is scored from 0 to 4, with higher scores indicating greater physical function.

Handgrip strength was measured three times in each side using a digital hand dynamometer (Seahan Corp., Masan, South Korea). The maximal grip strength was used for analysis.

Statistical analysis

Because cortical porosity was the main microarchitectural feature affected in diabetes in previous studies, we used the difference in cortical porosity previously reported between patients with diabetes with and without fractures (3.86% ± 1.30 versus 0.83 ± 0.13, respectively)[20] as the clinically significant difference. This resulted in a sample size of 20 in each group. This sample size has 80% power to detect a difference of 3.0% in cortical porosity at $p < .05$.

Variables are described as mean and standard deviation (normally distributed) or median and interquartile range (non-normally distributed). Normally distributed variables were compared using ANOVA followed by Scheffe post hoc test. Non-normally distributed variables were compared using the Kruskal–Wallis test. For these analyses, a $p < .05$ was considered significant. For non-normally distributed variables, the Mann–Whitney test was used for pairwise comparison with the Bonferroni correction for multiple comparison, resulting in a $p < .017$ as significant.

In the participants with T1D, the relationship between markers of nerve conduction assessment and DXA and HR-pQCT features were assessed using Spearman's rank correlation (non-normally distributed). The analyses were performed using IBM SPSS Statistics for Mac (version 25.0, IBM Corp., Armonk, NY, USA).

Results

Seventy-one potential participants were screened for this study, 10 were excluded according to inclusion and exclusion criteria, and 1 participant withdrew after the screening visit. Sixty participants were recruited: 20 participants T1DN+, 20 T1DN–, and 20 healthy controls (control). Table 1 shows the characteristics of the 60 participants. As expected, HbA1c was higher in diabetics compared with controls. Among the diabetic group, there was no difference in diabetes duration between the groups (T1DN+ 28.9 years ±10.6 and T1DN– 24.6 years ±15.5). Participants with T1D had had more fractures than participants without diabetes. In T1D patients, the mean age at diagnosis was 22.2 ± 12.0 years and the duration of disease ranged from 6 to 58 years (mean 26.5 ± 13.1 years). We used the mean HbA1c from clinical records (5 to 10 years) to estimate metabolic control (mean HbA1c), and it was worse in T1DN+ than T1DN– (Table 1). In 16 (40%) of the T1D patients, diabetes onset was before 20 years old.

DXA

aBMD measured by DXA at the femoral neck (FN), total hip (TH), and lumbar spine (LS; L_1 to L_4) was not different between the groups (Table 1). In the diabetic groups, FN and TH BMD correlated positively with nerve conduction velocity ($r = 0.481$, $p = .002$ and $r = 0.388$, $p = .015$, respectively). ASMI was not different between the three groups (Table 1).

Table 1. Study Population Characteristics, Neuropathy Assessment, DXA, Bone Turnover Markers, and Physical Tests Outcomes Reported as Mean (Standard Deviation) for Normally Distributed Variables and Median (Interquartile Range) for Non-normally Distributed Variables

	$T1DN^+$ (n = 20)	$T1DN^-$ (n = 20)	Control (n = 20)	*p* Value
Age (years)	47.7 (11.0)	49.6 (13.1)	49.1 (12.5)	.872
Height (cm)	172.6 (8.2)	171.4 (10.3)	170.6 (9.7)	.792
Weight (kg)	77.6 (18.4)	72.9 (12.0)	71.4 (10.7)	.358
BMI (kg/m^2)	25.9 (5.2)	24.8 (3.6)	24.4 (2.5)	.486
Tibia length (mm)	396.3 (25.7)	388.5 (29.5)	387.0 (30.1)	.549
Radius length (mm)	280.0 (21.9)	276.0 (23.0)	277.5 (21.1)	.850
HbA1c (mmol/mol)	70.2 (14.3)	62.5 (14.6)	34.7 (3.2)	<.001
Mean HbA1c (mmol/mol)	77.1 (17.5)	64.4 (11.4)	NA	.01
Diabetes duration (years)	28.9 (10.6)	24.1 (15.3)	NA	.108
TCNS	13.3 (5.7)	2.5 (1.7)	NA	<.001
DPNCheck conduction velocity	32.1 (17.9)	48.4 (4.2)	NA	<.001
DPNCheck amplitude	3.6 (2.3)	11.7 (6.3)	NA	<.001
Sex (female)	8	8	8	
Previous fractures (*n*)	13	10	5	.038
Falls in the last 6 months (*n*)	2	2	3	.851
Smoking (current/ex)	2/5	1/3	1/9	.269
LS *T*-score	−0.3 (−1.7, 0.5)	−0.7 (−1.7, 0.1)	−0.8 (−2.0, −0.2)	.597
FN *T*-score	−1.2 (−2.0, −0.5)	−0.7 (−1.5, 0.0)	−1.2 (−1.6, −0.6)	.181
TH *T*-score	−0.5 (−0.9, 0.2)	−0.2 (−0.7, 0.6)	−0.6 (−1.1, 0.0)	.423
ASMI (kg/h^2)	7.4 (1.3)	7.6 (1.2)	7.4 (1.4)	.853
PINP (ng/mL)	41.9 (29.4, 50.2)	38.1 (31.8, 51.2)	58.0 (47.1, 79.5)	.013
CTX-I (ng/mL)	.037 (0.033, 0.102)	.047 (0.033, 0.115)	.357 (0.039, 0.641)	.014
SPPB (score)	10.3 (2.1)	11.8 (0.4)	11.9 (0.4)	<.001
Hand grip (Kg)	28.5 (20.6–33.9)	28.4 (21.1–40.8)	35.1 (20.9–42.6)	.418

BMI = body mass index; HbA1c = hemoglobin A1c or glycosylated hemoglobin; TCNS = Toronto Clinical Neuropathy Score; LS = lumbar spine; FN = femoral neck; TH = total hip; ASMI = Appendicular Skeletal Muscle Mass Index; SPPB = Short Physical Performance Battery.

HR-pQCT results

All the participants were assessed at the wrist and ankle. Nine standard ultra-distal site radial scans were excluded because of movement artifacts (4 T1DN+, 4 T1DN–, and 1 control). Twenty-one exploratory 14% offset radius site scans were not available because of technical issues (movement artifact or the exploratory 14% offset site was out of the scanning area), namely 8 in the T1DN+ group, 7 in the T1DN– group, and 6 in the control group. One

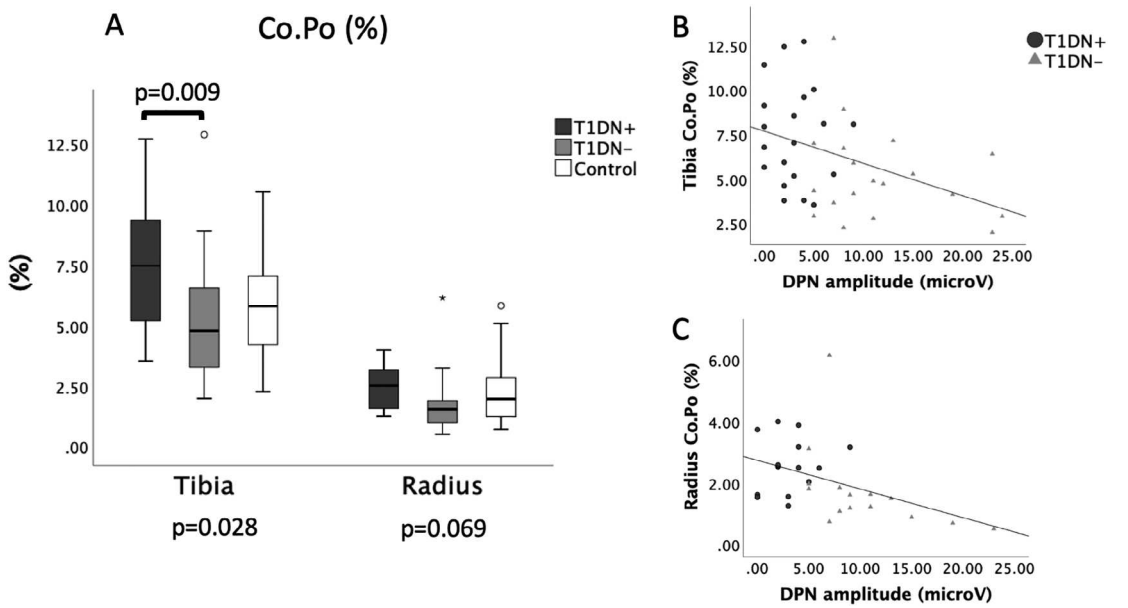

Fig 3. (*A*) Box and whisker plot of cortical porosity (%) at tibia and radius at standard ultra-distal site. (*B*) Correlation Co.Po (%) and nerve amplitude (μV) at the tibia ($r = -0.386$; $p = .015$) and (*C*) radius ($r = -0.484$; $p = .006$).

Table 2. Tibia and Radius Geometry, BMD, Microarchitecture and Estimate Biomechanical Parameters at the Standard Ultra-Distal Site Data Given as Mean (SD) or Median (IQR)

	Tibia				Radius			
	T1DN$^+$ (n = 20)	T1DN$^-$ (n = 20)	Control (n = 20)	p Value	T1DN$^+$ (n = 16)	T1DN$^-$ (n = 16)	Control (n = 19)	p Value
Geometry								
Total area (mm^2)	835 (152)	771 (119)	766 (144)	.227	358 (67.9)	343 (86.2)	338 (88.1)	.765
Trabecular area (mm^2)	697 (151)	627 (100)	627 (130)	.151	288 (62)	273 (77.3)	273 (80.4)	.81
Cortical area (mm^2)	131 (36.1)	139.6 (44.8)	135.4 (27)	.762	63.5 (15.3)	64.3 (18.2)	57.6 (14.6)	.4
Volumetric BMD								
Total vBMD (mg HA/cm^3)	296 (57.7)	318 (61.7)	303 (36.9)	.422	311 (60.9)	322 (64.6)	307 (50.5)	.737
Tb.vBMD (mg HA/cm^3)	182 (44.6)	188 (40.8)	172 (29.2)	.456	171 (52.6)	171 (37.1)	168 (36.5)	.968
Ct.vBMD (mg HA/cm^3)	845 (817–895)	901 (861–929)	892 (833–916)	.106	873.8 (842.4–895.8)	876.8 (850.4–904.8)	872.6 (816.2–895.2)	.577
Microarchitecture								
Tb.BV/TV[d]	.152 (0.037)	.156 (0.034)	.144 (0.025)	.457	.143 (0.044)	.143 (0.031)	.140 (0.030)	.969
Tb.N (1/mm)	1.96 (0.44)	1.95 (0.33)	1.8 (0.25)	.300	1.91 (0.33)	1.97 (0.27)	1.99 (0.25)	.716
Tb.Th (mm)[d]	.078 (0.014)	.080 (0.011)	.080 (0.008)	.805	.074 (0.016)	.072 (0.011)	.070 (0.011)	.629
Tb.Sp (mm)[d]	.409 (0.367, 0.528)	.448 (0.373, 0.505)	.494 (0.431, 0.540)	.288	.446 (0.384, 0.491)	.424 (0.389, 0.479)	.420 (0.399, 0.472)	.814
Ct.Th (mm)[d]	1.16 (0.31)	1.23 (0.39)	1.24 (0.21)	.660	.78 (0.18)	.81 (0.19)	.72 (0.16)	.334
Ct.Po (%)[d]	7.48 (5.19, 9.48)	4.8 (3.1, 6.64)[a]	5.81 (4.14, 7.36)	**.028**	2.52 (1.58, 3.18)	1.56 (0.94, 1.94)	1.98 (1.2, 2.86)	.069
Estimated biomechanical parameters								
Stiffness (kN/mm)	244 (58.5)	250 (67.9)	235 (54.4)	.749	98.9 (29.2)	96.6 (26.8)	88.7 (27.7)	.523
Failure load (kN)	12.3 (3)	12.5 (3.3)	11.8 (2.7)	.730	5 (1.5)	4.9 (1.3)	4.5 (1.4)	.571

Tb.vBMD = trabecular volumetric bone mineral density; Ct.vBMD = cortical volumetric bone mineral density; Tb.BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Ct.Po = cortical porosity.

[a]T1DN$^-$ compared with T1DN$^+$, p = 0.009.

[d]Derived measurement method[33].

Significant p values are shown in bold.

Table 3. Tibia and Radius Geometry, BMD, Microarchitecture, and Estimate Biomechanical Parameters at Exploratory 14% Offset Site Data Given as Mean (SD) or Median (IQR)

	Tibia				Radius			
Exploratory 14% offset site	T1DN$^+$ (n = 20)	T1DN$^-$ (n = 20)	Control (n = 19)	p Value	T1DN$^+$ (n = 12)	T1DN$^-$ (n = 12)	Control (n = 14)	p Value
Geometry								
Total area (mm^2)	559 (487, 642)	522 (470, 555)	544 (454, 595)	.438	534.3 (86)	531.2 (70.6)	502.2 (68.7)	.477
Trabecular area (mm^2)	380 (84.9)	344 (64.2)	348 (57.3)	.218	89.9 (25.5)	81 (22.9)	80.7 (27)	.573
Cortical area (mm^2)	178 (29.3)	180 (44.9)	180 (29.8)	.981	82.9 (8.8)	85.7 (17.4)	80.3 (16.4)	.637
Volumetric BMD								
Total vBMD (mg HA/cm^3)	414 (66.6)	437 (91.8)	419 (49.7)	.560	576.9 (539.4, 617.7)	589.0 (562.9, 690.4)	598.9 (541.4, 634.6)	.463
Tb.vBMD (mg HA/cm^3)	143 (39.3)	143 (33)[b]	118 (24.7)	**.015**	140.8 (50.6)	148.2 (41.2)[c]	101.3 (27.3)	**.009**
Ct.vBMD (mg HA/cm^3)	987 (958, 1003)	1001 (965, 1019)	1006 (962, 1021)	.529	1042.5 (1027.5, 1080.9)	1061.0 (1039.4, 1072.8)	1057.5 (1031.3, 1073.6)	.962
Microarchitecture								
Tb BV/TVd[d]	.119 (0.033)	.124 (0.027)[b]	.099 (0.021)	**.015**	.117 (0.042)	.123 (0.034)[c]	.084 (0.023)	**.009**
Tb.N (1/mm)	1.70 (0.41)	1.79 (0.33)[b]	1.47 (0.19)	**.010**	1.53 (0.41)	1.7 (0.25)[b]	1.31 (0.25)	**.009**
Tb.Th (mm)[d]	.071 (0.014)	.070 (0.013)	.067 (0.010)	.631	.077 (0.017)	.072 (0.015)	.064 (0.013)	.122
Tb.Sp (mm)[d]	.49 (0.45, 0.70)	.51 (0.42, 0.61)[b]	.60 (0.57, 0.66)	**.032**	.59 (0.50, 0.86)	.50 (0.47, 0.60)[c]	.65 (0.58, 0.92)	**.008**
Ct.Th (mm)[d]	1.94 (0.30)	2.01 (0.47)	2.01 (0.25)	.808	1.58 (0.18)	1.64 (0.23)	1.57 (0.2)	.632
Ct.Po (%)[d]	2.48 (1.2, 3.93)	1.96 (1.42, 2.95)	1.51 (1.1, 2.97)	.660	.56 (0.39, 1.06)	.41 (0.26, 1.07)	.56 (0.21, 1.00)	.660
Estimated biomechanical parameters								
Stiffness (kN/mm)	240.2 (43.3)	239.8 (58.7)	233.4 (42.3)	.886	100 (13.8)	99.1 (24.2)	95.0 (21.5)	.784
Failure load (kN)	12 (2.2)	11.9 (2.9)	11.6 (2.1)	.889	5.0 (0.7)	4.9 (1.2)	4.7 (1.1)	.794

Tb.vBMD = trabecular volumetric bone mineral density; Ct.vBMD = cortical volumetric bone mineral density; Tb.BV/TV = trabecular bone volume fraction; Tb.N = trabecular number; Tb.Th = trabecular thickness; Tb.Sp = trabecular separation; Ct.Th = cortical thickness; Ct.Po = cortical porosity.

[b]T1DN– compared with controls Tb.vBMD Tibia p =.024 Radius p =.017; Tb.BV/TV Tibia p =.023 Radius 0.017; Tb.N Tibia p =.014 Radius p=.010; Tb.Sp Tibia p=.015 Radius 0.001.

For Tb.SP, non-parametric test was used ($p < .017$).

[d]Derived measurement method[33].

Significant p values are shown in bold.

exploratory 14% offset site at the tibia (control) was not included because of movement artifacts.

At the standard ultra-distal site, tibial cortical porosity was 56% higher in T1DN+ compared with T1DN– ($p = .009$) (Fig. 3) and tended to be higher in T1DN+ compared with controls, but it did not reach statistical significance. There was also a trend to similar results at the radius (Fig. 3). Because cortical porosity is highly sensitive to reduced image quality, we reran the analysis including only high-quality images (grades 1 and 2) and found similar results. There were no other significant differences among the three groups at the radius or tibia (Table 2). Tibia total and cortical density and cortical TMD correlated positively with nerve conduction velocity ($r = 0.416$, $p = .008$; $r = 0.408$, $p = .01$; and $r = 0.365$, $p = .022$, respectively). Cortical porosity correlated negatively both with nerve conduction velocity and amplitude at the tibia ($r = -0.358$, $p = .025$ and $r = -0.386$, $p = .015$, respectively) and radius ($r = -0.446$, $p = .012$ and $r = -0.484$, $p = .006$) (Fig. 3). At the tibia, cortical porosity also correlated positively with TCNS ($r = 0.347$, $p = .028$).

At the exploratory 14% offset site, we found favorable trabecular microarchitecture when comparing T1DN– and control both at the radius and tibia in a consistent pattern (Table 3, Fig. 4). We found higher trabecular volumetric BMD (Tb.vBMD; 46% higher at the radius, $p = .017$; 25% higher at the tibia, $p = .024$) and trabecular number (Tb.N; 30% higher at the radius, $p = .010$; 22% higher at the tibia, $p = .014$). Trabecular thickness was not different between the groups (Fig. 4). There was a trend for T1DN+ to follow the same pattern, but it did not reach statistical significance. No significant differences in any HR-pQCT parameters at the exploratory 14% offset site were found between diabetic groups. There were no differences in cortical features or estimated biomechanical parameters among the three groups at the exploratory 14% offset site.

BTM results

PINP and CTX were lower in both T1DN+ and T1DN– compared with controls. PINP was 34% ($p = .006$) and 28% (non-significant) lower in T1DN– and T1DN+ compared with controls, whereas CTX was 87% ($p = .016$) and 90% ($p = .011$) lower, respectively (Fig. 5). No difference was found between diabetic groups (T1DN+ and T1DN–).

Physical performance

T1DN+ participants had worse performance in the SPPB but not at the handgrip test than T1DN– and healthy controls (Table 1). Four of 20 T1DN+ had poor performance according to the revised European consensus on definition and diagnosis of

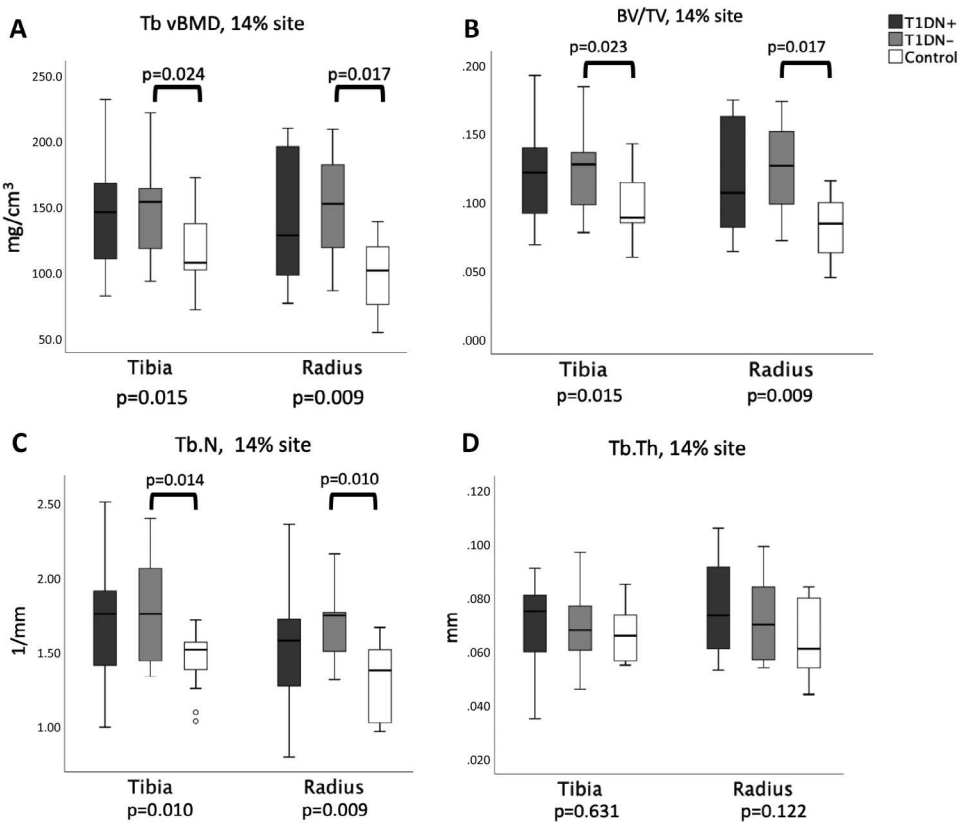

Fig 4. Box and whisker plot of (*A*) trabecular volumetric BMD (Tb.vBMD), (*B*) trabecular bone volume (BV/TV), (*C*) trabecular number (Tb.N), and (*D*) trabecular thickness (Tb.Th) at radius and tibia at 14% site.

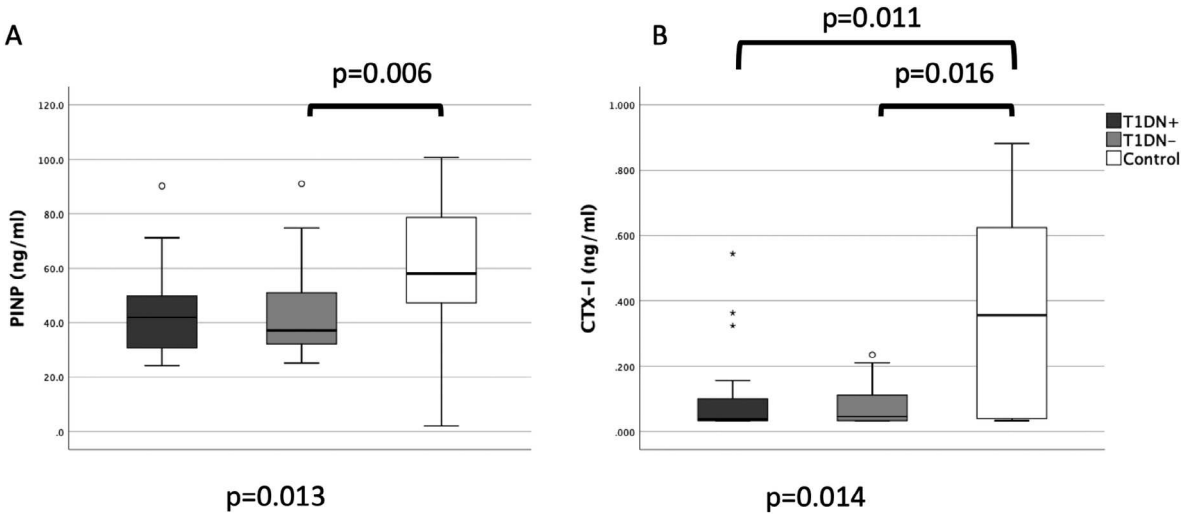

Fig 5. Box and whisker plot of PINP (*A*) and CTX (*B*) in the three groups.

sarcopenia.[42] T1DN– performance was similar to the control group. ASMI was not different between the groups (Table 1).

Discussion

This is the first study to investigate the effect of DPSN on the skeleton in T1D. Cortical porosity was higher in T1DN+ compared with T1DN– at the tibia standard ultra-distal site. Cortical porosity at the tibia correlated positively with TCNS and negatively (both at the radius and tibia) with nerve conduction amplitude and velocity. Conversely, total and cortical tibia density and TMD correlated positively with nerve conduction velocity. At the exploratory 14% offset site (less distal), we found favorable trabecular microarchitecture, both at the radius and the tibia in T1DN– in a consistent pattern. There was a trend toward the same pattern in T1DN+. We found low bone turnover as both CTX (T1DN+ and T1DN–) and PINP (T1DN–) were lower in participants with diabetes compared with controls. aBMD measured by DXA was not different between the three groups, but proximal femur BMD was positively correlated to nerve conduction velocity in the diabetic groups. Despite no difference in ASMI, T1DN+ had worse performance in the SPPB but not in handgrip strength compared with T1DN– and control.

Our findings suggest that neuropathy is associated with increased cortical porosity. There is ex vivo and clinical evidence for a neuronal control of bone remodeling.[21,22] Recently, a histomorphometry study in humans with high bone turnover showed clear anatomical association of nerves and bone remodeling surfaces and a high density of nerve profiles in intracortical pores. The authors claim that this anatomical link between innervation and bone remodeling sites suggested a role for innervation in bone remodeling.[22] In participants with T1D, we found negative correlations between nerve conduction amplitude and velocity and cortical porosity at the tibia and radius. In addition, TCNS correlated positively with tibial cortical porosity, suggesting that more severe neuropathy is associated with higher cortical porosity. We also found positive correlations between proximal aBMD (both FN and TH), and peripheral total and cortical vBMD at the tibia and nerve conduction velocity. Taken together, these findings suggest a positive association of nerve function on cortical bone density.

We found low bone turnover and preserved trabeculae in T1D, and these findings might be linked. A number of studies have reported low bone turnover associated with both T1D and T2D,[13,14,43] suggesting a role for hyperglycemia. In vitro studies have shown that chronic hyperglycemia inhibits osteoclast[44] and osteoblast differentiation and activity.[45] The glucose can also bind to proteins, leading to the formation of advanced glycation end products (AGEs). Hyperglycemia promotes the formation of AGEs, especially in long-lived tissue proteins such as collagen. In vitro studies have shown that AGEs also decrease osteoclast differentiation and activity and osteoblast activity.[46,47] Therefore, there is evidence for both direct and indirect effects of hyperglycemia in the skeleton. Shanbhogue and colleagues also reported lower BTM in T1D but, in contrast to our study, they reported lower vBMD (lower total and trabecular BMD and lower trabecular thickness at radius and tibia) and lower estimated biomechanical parameters when comparing T1D participants with and without MVD.[13] Noteworthily, the study design was different; the groups were not matched and there was a sex imbalance, with more males in the group without MVD. In addition, they reported the data for overall MVD, while we focused on neuropathy.

Limitations of the HR-pQCT method could contribute to the inconsistent findings at the standard ultra-distal and exploratory 14% offset sites. Although we report favorable microarchitecture at the tibia and radius at the exploratory 14% offset site for T1DN–, we did not find the same pattern at the standard ultra-distal site. Shanbhogue and colleagues have reported that variation in bone length introduces a systematic error in the estimation of some HR-pQCT–derived bone variables such as trabecular number.[48] We speculate that these errors could have influenced our results at the standard ultra-distal site. In addition, we reported increased cortical porosity at the standard ultra-distal site in the T1DN+ group but not at the exploratory 14% offset site. Two features might have influenced these findings. First, cortical porosity was lower at the exploratory 14% offset site; median 1.51–2.48% at the exploratory 14% offset site compared with 4.8–7.48% at the standard ultra-distal site at the tibia, and

0.41–0.56% and 1.56–2.52%, respectively, at the radius. It is possible that at the exploratory 14% offset site, most of the pores were below the threshold of detection. In addition, we claim that neuropathy might also have influenced the results. Because peripheral neuropathy is a length-dependent process, we speculate that its effects would be more evident more distally.

Previous studies have also reported inconsistent results in microarchitecture in diabetes.[13–18] In the cortical compartment, both increase[14,16,17,20] and decrease[11,15] in cortical porosity have been previously reported in T2D. Our cortical findings agree with previous data that reported an increase in cortical porosity in T2D at the standard ultra-distal site.[16–18] Although Shanbhogue and colleagues reported higher cortical porosity in T2D with MVD,[14] in de Waard and colleagues, the presence of MVD was not associated with any bone parameters.[15] The previous study in T1D found no differences in cortical porosity.[13] In the trabecular compartment, most studies in T2D reported no findings, while two cohorts reported favorable findings.[15,19] A cohort of 954 elderly women, including 99 with T2D, 60% of them with early disease, showed that T2D was associated with favorable trabecular microarchitecture at the standard ultra-distal site, in a similar pattern to what we found at the exploratory 14% offset site, with higher trabecular BV/TV and trabecular number.[19] In addition, data from The Maastricht Study including 410 (radius) and 198 (tibia) participants of mean age 58 years, 51% of females reported higher Tb.N both at the radius and tibia and no influence of MVD.[15] In T1D, unfavorable trabecular findings were reported when comparing participants with and without MVD, as mentioned above. Our results at the exploratory 14% offset site are similar to the favorable findings in T2D at the standard ultra-distal site in bigger cohorts. In contrast with the previous study in T1D, we found no differences between the diabetic groups, either at the standard ultra-distal or the exploratory 14% offset site.

Favorable trabecular findings have also been reported in histomorphometry in T2D.[49] Recently, Andrade and colleagues compared histomorphometry in premenopausal T2D women with good (n = 10, HbA1c < 7%) and poor metabolic control (n = 16, HbA1c > 7%) to age- and race-matched controls without diabetes.[49] The authors reported greater BV/TV in T2D with good control compared with the non-diabetic control group and borderline findings in the poor control group (p = .05). Furthermore, there was greater Tb.N and lower trabecular separation in both T2D groups compared with non-diabetics regardless of the metabolic control. There was a negative correlation between HbA1c and features of osteoid function, such as osteoid thickness and osteoid surface.[49] These findings suggested that mild hyperglycemia was associated with preserved trabecular structure and reduced bone formation, similarly to our findings in T1DN-.

Interestingly, although significant differences were found on cortical porosity at the standard ultra-distal site and on trabecular compartment at the exploratory 14% offset site, there were no differences in FE results. We speculate that the differences found in each compartment were not big enough to result in significant differences in biomechanical parameters. Furthermore, it is important to consider that we have used standard material properties for the FE calculation, and it is possible that bone material properties could be altered in diabetes.[50,51]

The risk of fractures is increased in T1D and despite the increased cortical porosity in T1DN+, the favorable trabecular findings in T1DN– might seem like a paradox. This paradox suggests a role for non-skeletal features on this increased risk. The vast majority of the non-spine fractures are attributed to falls.[52] There is evidence that hypoglycemia and neuropathy increase the risk of falls.[53,54] Older women with diabetes have an increased risk of falls and the risk is higher in insulin users.[55] Shah and colleagues investigated falls in T1D and reported a high frequency among middle-aged and older adults with T1D.[54] Severe hypoglycemia was associated with a threefold increase in the risk of falls and peripheral neuropathy with a twofold increase in this risk. The presence of neuropathy and tripping over an uneven surface were the most common factor related to falls reported by participants.[54] In our study, T1DN+ had worse performance in SPPB. This test battery assesses balance, gait, and lower limb strength[41] and was associated with an increased risk of falls.[56] Balance and gait are complex tasks. Information received from the vestibular, visual, and somatosensory systems are combined and the final control is mediated through the motor system.[57] Neuropathy might negatively affect the somatosensory and motor systems and increase the risk of falls. Cohort studies have shown worse physical performance in elderly people with diabetes, partially mediated by a decrease in peripheral nerve function.[19,25,26]

In addition, muscle function can also be affected in diabetes. T1D children with poor glycemic control had lower grip strength than non-diabetic children.[58] In adolescents, dynamic muscle function evaluated by jumping mechanography showed lower relative muscle power and force in T1D compared with the reference.[59] A Japanese study assessed participants with T1D (42 to 75 years) without severe neuropathy and reported poor performance, compatible with sarcopenia according to the Asian Working Group for Sarcopenia criteria, including lower muscle mass and handgrip and lower limb strength.[60] We found no difference on ASMI or physical test performance between T1DN– and controls, but T1DN+ participants had worse performance at SPPB. In addition, 4 T1DN+ participants had poor performance on physical tests, suggesting sarcopenia. Therefore, neuropathy was associated with worse lower limb muscle function despite no difference in muscle mass, suggesting an important role for ataxia and muscle weakness.

We found no differences in aBMD between the groups. Data in aBMD in T1D is conflicting,[5,61] but a meta-analysis reported a decrease in aBMD.[5] We speculate that our sample size was too small to detect any difference, especially between groups matched for weight.

This study has limitations. We were not able to match participants individually to important confounders such as age, height, and body mass index, but there were no differences between the groups. We used a point-of-care device to assess nerve conduction, but it has been previously validated against the gold standard method.[62] We assessed only the sural nerve, whereas, ideally, nerve conduction studies should test multiple nerves to confirm polyneuropathy; however, clinical features were also assessed by the TCNS. We included an exploratory 14% bone length site that has not been validated, but other less distal sites have been previously described,[19,20] and the analysis showed interesting results. At the exploratory 14% offset site, cortical bone is thicker and therefore more susceptible to the effect of beam hardening. We did not assess bone material properties. We used SPPB to assess muscle function, and the test provides limited detail in the contribution of somatosensory and motor function in the physical function performance. We assessed participants with T1D. Because diabetic neuropathy also affects T2D, we speculate that similar associations between neural dysfunction and skeletal features could affect this population, but further studies should be done to investigate T2D.

In summary, we report an increase in cortical porosity associated with DSPN but favorable trabecular microarchitecture in T1DN–. We speculate that nerve dysfunction and low bone turnover have influenced these results. We did not assess bone material properties, but our findings suggest that bone structure contributes but does not fully explain the higher fracture risk in T1D. In addition, T1DN+ had worse performance in tests that assess not only muscle strength but also balance and gait. Our findings suggest that the increase in the risk of fractures in T1D is multifactorial and both skeletal and non-skeletal features are involved.

Disclosures

JSW has received speaker's honoraria from Eli Lilly and Sandoz; grant funding from Alexion and Immunodiagnostic Systems; donation of drug from Eli Lilly, Prostrakan (Kyowa Kirin), and Consilient for clinical studies; donation of assay kits from Biomedica; and consulting fees from Shire, Mereo Biopharma, Kyowa Kirin, UCB Pharma, and PharmaCosmos. RE receives consultancy funding from IDS, Sandoz, Nittobo, Samsung, Haoma Medica, CL Bio, Biocon, Lyramid, and Viking and grant funding from Nittobo, Roche, and Alexion. All other authors state that they have no conflicts of interest.

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Authors' roles: Study design: TV and RE. Study conduct: TV, JSW, and DS. Data collection: TV and MP. Data analysis: TV and MP. Data interpretation: TV and RE. Drafting manuscript: TV. Revising manuscript content: TV, RE, MP, JSW, and DS. Approving final version of manuscript: all authors. TV takes responsibility for the integrity of the data analysis.

Author contributions: TV: Conceptualization,Methodology, Formal analysis, Investigation, Writing-Original Draft, Writing-Review & Editing; MP: Formal analysis; investigation; writing-review & editing. JSW: Supervision; writing-review & editing. DS: Methodology; supervision; writing-review & editing. RE: Conceptualization; funding acquisition; supervision; writing-review & editing.

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